

Investigating the Changes in Fish Behavior in Response to Anthropogenic Sound

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Chapter 1

A brief background on anthropogenic sound and fish hearing

Fish behavior is heavily dependent on biotic and abiotic factors that range from anthropogenic sounds that mask or impede fish communication (Clark et al., 2009; Kunc et al., 2016; Nikolich et al., 2021) to intra and interspecific interactions that shift mating, foraging, habitat acquisition and dominance hierarchies (McCormick & Weaver, 2012; Louhi et al., 2013). Fish in varying environments also depend on the aquatic soundscape to locate prey, aid in predator avoidance behaviors, and promote social interactions (Fay, 2009; Brintjes & Radford, 2013; Mensinger et al., 2016; Rountree et al., 2018). Many freshwater and marine species, such as the green sunfish (*Lepomis cyanellus*) and oyster toadfish (*Opsanus tau*), produce grunts during active courtship by males and rely on these vocalizations to attract mates and maintain their life history (Gerald, 1971; Mischke et al., 2007; Van Wert & Mensinger, 2019). Since these individuals depend on the soundscape to promote reproductive behavior, they are susceptible to anthropogenic sounds that may mask their vocalizations or decrease their auditory sensitivity levels (Nissen et al., 2019; Rogers et al., 2020; Rountree et al., 2020). Although the effects of anthropogenic sound on fishes is a major concern as sound may mask vocalizations important for everyday life or cause other behavioral changes, most studies have been conducted under a laboratory setting that can affect fish behavior and acoustic stimuli (Oldfield, 2011; Benhaïm et al., 2012; Duncan et al., 2016; Popper & Hawkins, 2019). To fully understand of the effects of anthropogenic sound on fish, in-situ non-invasive monitoring techniques need to be implemented to observe fish behavior under natural conditions.

Aquatic Soundscapes

Aquatic soundscapes are composed of physical, biological, and anthropogenic sounds (Pijanowski et al., 2011; Erbe et al., 2016). Natural sounds, which contribute to

the ambient soundscape in a given environment, are derived from physical processes such as wind, waves or ice break up as well as sounds produced by aquatic animals for communication, navigation, reproduction, and foraging (Erbe et al., 2016; Gottesman et al., 2018; Putland & Mensinger, 2020). In marine and freshwater environments, the ambient soundscape can widely vary based on location, depth, habitat, time of day, and lunar phase (Nedelec et al., 2015; Butler et al., 2016; Larsen & Radford, 2018). In a submarine canyon in Western Australia, whales seasonally dominate the soundscape at low (15 – 100 Hz) and mid (200 – 400 Hz) frequencies while fish and invertebrates dominate high frequencies (1800 – 2500 Hz) at night (Erbe et al., 2015). In the Adriatic Sea, snapping shrimp are the primary biological sound producers (above 620 Hz frequencies) in the early morning and late evenings, whereas fish vocalizations dominated at lower frequencies (< 620 Hz) during the night (Pieretti et al., 2017). Considering the variability of aquatic soundscapes, the abundance of knowledge on marine soundscapes is evident when compared to the lack of data for sound sources within freshwater habitats (Rountree et al., 2018).

Freshwater acoustic research mainly focuses on identifying and characterizing the bioacoustics of individual species ranging from amphibians (Narins & Feng, 2006; Bee, 2012) to fish (Mann et al., 2007; Cott et al., 2013). Only recently has freshwater research investigated the ambient soundscape of freshwater lakes (Putland & Mensinger, 2020) and the effects of anthropogenic sound on freshwater fish behavior in the wild (Pieniazek et al., 2020). A recent study on the ambient soundscape of 15 freshwater lakes in Minnesota, USA, used passive acoustic monitoring to determine that the median broadband sound pressure level was significantly lower in winter (57.2 dB re 1 μ Pa)

during complete ice cover compared to summer (66.7 dB re 1 μ Pa) and that anthropogenic sounds from ice augers and motorboats directly overlapped with the frequency range used by many aquatic animals for communication (Putland & Mensinger, 2020).

Anthropogenic sound has been found to decrease foraging in wild, freshwater fish (Pieniazek et al., 2020) and in lotic systems with naturally noisier ambient soundscapes due to water movement from riffles, shoals, and waterfalls. Additionally, species that rely on the soundscape for communication during courtship, such as the Blacktail Shiner (*Cyprinella venusta*), were directly impacted by nearby development and road traffic masking their vocalizations (Holt & Johnston, 2015). Despite more concentrated efforts on freshwater soundscape research, the need to understand the connection between different organisms (fish, amphibians, mammals, reptiles, arthropods) and their environment becomes increasingly important with greater anthropogenic stressors (Greenhalgh et al., 2020).

Anthropogenic Sound

The soundscapes of many marine and freshwater environments have been changing at an alarming rate due to the expansion of transportation, development, and resource extraction and advances in economic growth are positively correlated with an increase in anthropogenic stressors on terrestrial and aquatic ecosystems (Buxton et al., 2017; Cox et al., 2018). Since roughly 40% of the global population live within 100 km of the coastline, aquatic regions are especially susceptible to changes in the ambient soundscape (Cohen et al., 1997; Small & Nicholls, 2003; Cox et al., 2018). In nearshore tropical marine environments with extensive human impacts, habitat degradation can diminish unique, natural soundscapes important for fishes and other aquatic species

(Butler et al., 2016). In freshwater lakes, anthropogenic sounds produced by ice augers in winter and motorboats in summer (100 – 1000 Hz) can increase the intensity of the soundscape by 10 dB and overlap with the frequency range of biological sounds (300 – 1000 Hz) in the environment (Putland & Mensinger, 2020). Anthropogenic sound, in turn, can lead to behavioral changes, masking, increased cortisol, hearing damage or injury in aquatic animals (Clark et al., 2009; Kunc et al., 2016).

An increasing concern, especially regarding marine and freshwater protected areas, is the lack of regulations surrounding anthropogenic sound pollution since underwater sound has no boundaries. In Rockfish Conservation Areas in British Columbia, Canada, Pacific rockfish (*Sebastes spp.*) experience vessel sounds that can mask rockfish communication (Nikolich et al., 2021). In the Parque Nacional Arrecifes de Cozumel in Mexico, cruise ships and small motorboat traffic overlapped with splendid toadfish (*Sanopus splendidus*) vocalizations leading to high susceptibility of acoustic masking and a reduction in communication space which can negatively impact reproductive success (Pyc et al., 2021). Additionally, if fish are exposed to sound at high intensity, they can experience temporary or permanent hearing damage. In fathead minnows (*Pimephales promelas*), a white noise exposure has been found to significantly impact the auditory threshold in its most sensitive hearing range (800 – 2000 Hz) for over 14 days (Scholik & Yan, 2001) and in extreme cases, close proximity to intense sounds such as pile driving sources or explosions can cause physical damage to tissues and organs or lead to mortality (Yelverton et al., 1975; Popper & Hastings, 2009; Putland et al., 2019). However, the effects of anthropogenic sound on fishes directly depend on their hearing capabilities.

Sound Detection & Fish Hearing

It is important to consider the basic physics behind underwater sound to understand the differences in fish hearing capabilities. In general, sound waveforms travel through a medium by a transfer of energy (Urick, 1983). During this movement, alternating pressure deviations cause scalar and omnidirectional localized compression and refraction known as sound pressure. Particle motion, which is a vector that relates to the directionality of the propagating wave, is due to the vibration of particles back and forth transmitting the oscillatory motion to neighboring particles and dominates the hearing mechanisms of fish (Larsen & Radford, 2018; Putland et al., 2019). The basic inner ear of fish is composed of semi-circular canals and otolith end organs (sacculi, lagena and utricle). While the semi-circular canals are needed for maintaining equilibrium orientation, the otoliths are used for hearing and detection of linear acceleration (Rogers & Cox, 1988; Popper & Fay, 1997). Since fish are the same density as the surrounding medium, as they move in motion with the sound field, the denser otoliths lag behind and deform the neighboring sensory hair cells (Putland et al., 2019). This differential movement between the hair cells and the otolith depolarizes hair cells to release an excitatory neurotransmitter increasing afferent nerve firing, which is ultimately interpreted by the brain as sound (Fay & Popper, 1975).

Most fish species rely primarily on particle motion to detect sounds between 50 – 1000 Hz, however, some fishes have pressure detecting mechanisms such as a swim bladder connected directly or in close proximity to the inner ears that allows for a transduction mechanism to convert received sound pressure into particle motion that the inner ears can then detect (Sand & Hawkins, 1973; Fay & Popper, 1975). In otophysans,

Weberian ossicles link the swim bladder to the inner ear (Weber, 1820). As the swim bladder vibrates due to changes in sound pressure, this linkage transmits these vibrations to the inner ear and allows for higher frequency hearing ranges (up to 5,000 Hz) (Popper & Fay, 2011; Putland et al., 2019). Otophysan fishes account for roughly 64% of freshwater species and are known to be more susceptible to anthropogenic sound, making it especially important to understand the effects of sound on freshwater fish in numerous environments (Nelson et al., 1994; Briggs, 2005).

Non-invasive Monitoring Techniques

A variety of non-invasive monitoring techniques can be used to investigate the effects of anthropogenic sound on freshwater fish behavior. Passive acoustic monitoring (PAM) has been widely used in marine studies to describe fish vocalizations and better understand the aquatic soundscape, however, it is a relatively unexplored approach in monitoring freshwater environments (Rountree et al., 2006). Some advantages of PAM include its non-invasive approach, the ability to record remotely and autonomously over long time periods, and the capability of providing information on diversity and ecosystem health (Baumgartner et al., 2018; Desjonqueres et al., 2019). Recently, PAM was used to determine the baseline ambient soundscape in 15 freshwater lakes in Minnesota, USA (Putland & Mensinger, 2020) and to characterize the mating calls of freshwater *Prochilodus magdalenae*, which produce vocalizations during reproduction and allow for spawning ground identification (Munoz-Duque et al., 2021). By using PAM to monitor the soundscape and identify vocalizations from various aquatic animals, it is possible to determine the overlap and potential masking by anthropogenic sounds produced in or around freshwater ecosystems.

To further understand the effects of anthropogenic sound on fish, underwater video can be used to non-invasively monitor in-situ behavior. Underwater videos are associated with lower field costs and reduced diver presence, although they can be constrained by poor visibility and limited field of views (Cappo et al., 2003; Watson et al., 2005; Langlois et al., 2006). Underwater videos have also been suggested to be a better management tool for species such as those in the *Sparidae* family that are diver averse and important to recreational fisheries (Lowry et al., 2012). Previously, bait has been used in many underwater video studies to attract a substantial number of individuals to the area to investigate the effects of sound or other external stimuli on foraging behavior (Mensing et al., 2018; Pieniazek et al., 2020), but it is unclear whether bait is necessary to attract fish under more natural conditions in freshwater lakes. Further research is required to determine whether underwater videos and passive acoustic monitoring can be used to effectively and non-invasively observe changes in freshwater fish behavior in response to sound in field experiments.

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Chapter 2

The effect of boat sound on freshwater fish behavior in public (motorized) and wilderness
(nonmotorized) lakes

Preface

Freshwater lake soundscapes yield crucial information regarding biological, geological, and anthropogenic activity, yet is a relatively unexplored area of study. These soundscapes are particularly important to aquatic life that may use sound to navigate, find food, avoid predators and communicate. Further research is required to understand how aquatic species, such as native fishes, are impacted by increased anthropogenic interference. Many wilderness lakes restrict the use of motorized boats and equipment providing an opportunity to compare fish behavior in the presence and absence of anthropogenic sound. Underwater videos and passive acoustic monitoring were used to evaluate fish behavior under different soundscapes in the upper Midwest United States: John Lake (nonmotorized, Boundary Waters Canoe Area Wilderness, MN), Rush Lake (nonmotorized, Huron Mountain Club, MI) and Caribou Lake (motorized, Duluth, MN). Intermittent short and long anthropogenic sound playback experiments showed behavioral changes in bluegills (centrarchids), bluntnose minnows (cyprinids), mimic shiners (cyprinids) and yellow perch (percids) fishes. Overall, cyprinids in wilderness lakes were the most responsive to boat sound 35.8 to 52.2 dB above ambient sound levels, with bluegills in the public lake more likely to remain in the area during longer duration sound stimuli. Taken together, these results indicate that behavioral response are species specific and depend on environmental variables such as anthropogenic exposure and fishing pressure.

Introduction

Underwater soundscapes can influence the composition of a diverse array of aquatic communities and are important for aquatic organisms that rely on hearing for

orientation, prey detection, predator avoidance, social interactions and other behavioral responses (Cotter, 2008; Fay, 2009; Bruintjes & Radford, 2013; Mensinger et al., 2016; Rountree et al., 2018). However, underwater soundscapes are increasingly subjected to anthropogenic sounds (Koehn et al., 2011; Pratchett et al., 2011; Radford et al., 2014; Arthington et al., 2016; Poikane et al., 2017; Popper & Hawkins, 2019; Rountree et al., 2020). Motorized watercraft, nearshore construction, seismic testing and urbanization have led to an increase in background sound and can have detrimental effects on aquatic organisms (Popper et al., 2005; Kuehne et al., 2013; Putland & Mensinger, 2020). Despite numerous studies investigating the effects of anthropogenic sound on marine organisms, relatively fewer in situ studies have observed the effects of sound exposure on fish behavior in freshwater lakes, especially comparing areas subject to anthropogenic activity with wilderness environments that prohibit the use of motorized watercraft and vehicles (Rountree et al., 2018; Putland & Mensinger, 2020).

Recent studies on small, freshwater lakes in Minnesota, USA, revealed a relatively pristine year-round soundscape that may provide acoustic niches for aquatic animals in the absence of human activities (Putland & Mensinger, 2020). Seasonal variation in sound pressure levels (100-12,000 Hz) in these lakes was observed with significantly lower winter levels (57.2 dB re 1 μ Pa) compared to summer (66.7 dB re 1 μ Pa), which was attributed to a decrease in low frequency wind sounds due to ice cover and relatively less recreational activities (Putland & Mensinger, 2020). However, anthropogenic sounds (10-1000 Hz) were present year round, including shoreline construction/activity, motorized watercraft transiting open water, gas powered ice augers and/or snowmobiles operating on ice covered lakes which increased sound intensity and

overlapped the frequency range (300-1000 Hz) used by many aquatic animals for communication (Clark et al., 2009; Putland & Mensinger, 2020). Acoustic monitoring in numerous lakes and rivers in the eastern US also found that increased sound levels negatively influenced the biological diversity due to potential masking, suppression of sound production, or avoidance of regions with higher ambient sound levels (Rountree et al., 2020). Further research is needed to understand the impact that anthropogenic sound has on freshwater environments.

There is notable variation in the structures of the fish auditory organs in the inner ear among the 30,000+ extant fish species that influences hearing sensitivity (Popper & Fay, 2011; Putland et al., 2018). The ancestral and most common method of fish hearing is mediated through particle motion during inertial stimulation of the otolith organs resulting in frequency sensitivities of approximately 100 to 1,000 Hz (Fay & Popper, 1974; Popper & Fay, 2011). However, otophysan fishes (i.e. goldfish, catfish, minnows) have evolved Weberian ossicles that connect the swim bladder to the inner ear, enabling sound pressure detection and enhancing sensitivity and frequency detection over 3,000 Hz range (Fay & Popper, 2011). To correlate the effect of anthropogenic sound on fish behavior, it is necessary to understand the hearing capabilities of the fishes as behavioral responses to sound may differ in varying species.

Previous research primarily has focused on investigating changes in fish behavior to anthropogenic sound in laboratory studies, however these studies are challenging as captive fish may react differently than wild types (Oldfield, 2011; Benhaïm et al., 2012) and small tanks can affect the acoustic stimuli (Duncan et al., 2016; Popper & Hawkins, 2019). Recent studies have begun to investigate the impacts of anthropogenic sound on

wild fish populations (Jacobsen et al., 2014; Mensinger et al., 2016), including freshwater species (Jacobsen et al., 2014; Bolgan et al., 2016; Mickle & Higgs, 2017; Pieniazek et al., 2020). Motorized watercraft can be a dominant contributor of anthropogenic sound in aquatic environments and playbacks of these sounds often have been used to trigger fish behavioral responses (Whitfield & Becker, 2014; Holmes et al., 2017; Mensinger et al., 2018). For example, boat sound reduced foraging behaviors in captive black bullhead catfish (*Ameiurus melas*), a behavior consistent with similar observations in wild, Cyprinid freshwater fish (Pieniazek et al., 2020). Magnhagen et al. (2017) also found that cyprinid roach (*Rutilus rutilus*) decreased foraging attempts in response to boat motor sound, while the non-otophysan European perch (*Perca fluviatilis*) gradually increased feeding regardless of a noise stimulus. These studies suggest that fish behavior in response to sound is species specific and dependent on hearing capabilities, as cyprinids exhibited greater behavioral changes compared to fishes with lower hearing sensitivities.

Non-invasive research methods, such as passive acoustic or underwater video monitoring, can be used to investigate fish reactions to sound under natural conditions while baited underwater videos (BUVs) can attract and monitor fish in-situ while removing potential interference by boats or diver presence (Boom et al., 2014). However, video footage can be constrained by poor visibility, limited field of view and frequent battery recharging (Cappo et al., 2003; Watson et al., 2005; Castañeda et al., 2020) and the presence of the bait may impact behavior. BUVs have been successfully used to determine that Australian snapper (*Pagrus auratus*) populations respond differently to human generated sound and their density is greater in marine reserves compared to adjacent non-protected areas (Willis & Babcock, 2000; Mensinger et al., 2018). BUVs

have also helped determine the diversity of fish assemblages in seagrass habitat (French et al., 2021) and the higher diversity and abundance of sharks in protected (non-fished) marine regions (Clementi et al., 2021). Although underwater video experiments are utilized in a variety of marine environments, in contrast, few freshwater studies have used underwater video to monitor fish behavior in response to sound in the wild.

Many wilderness areas restrict the use of motorized activity and provide an excellent forum to compare fish behavior in lakes frequently impacted by anthropogenic sound to relatively pristine soundscapes. The aim of this study was to use non-invasive monitoring to compare how freshwater fish respond to anthropogenic sound in wilderness (motorized watercraft and vehicles prohibited) and public access (motorized watercraft and vehicles permitted) lakes. Underwater video recordings were conducted in Rush lake on the Huron Mountain Club property, a privately owned club containing remote, wilderness lakes in the Upper Peninsula of Michigan, John lake in the Boundary Waters Canoe Area Wilderness in northern Minnesota, and Caribou lake, a public access lake near Duluth, Minnesota. Fish in each lake were exposed to sound playback to replicate motorized watercraft during summer months. Based on previous studies, we expected fish in protected, wilderness lakes to display “bolder” behaviors and be less likely to leave the video frame in response to sound, especially during baited trials (Magnhagen et al., 2017; Pieniazek et al., 2020). We also expected otophysans to display more cautious behavior during a boat noise exposure due to greater hearing sensitivities than non-otophysans (Nissen et al., 2019).

Methods

Experiments were conducted at three freshwater lakes that differed in anthropogenic activity, but were similar in size, depth, and fish species. The study sites included Rush Lake (46.884140 °N, -87.907323 °W), a private lake located within the Huron Mountain Club lands in the Upper Peninsula of Michigan, John Lake (48.068456 °N, -90.057764 °W), a wilderness lake in the Boundary Waters Canoe Area Wilderness (BWCAW) in northern Minnesota and Caribou Lake (46.896980 °N, -92.322751 °W), a public access lake approximately 15 miles northwest of Duluth, MN (Fig.1). All trials were conducted within 500 m of the access point (indicated by the listed coordinates) for each lake. Deployment sites were accessed using a 5.6 m Kevlar canoe (Caribou Lake and John Lake) or a 3.7 m wooden rowboat (Rush Lake).

Caribou Lake (Fig. 1a) is a 2.2 km² public lake with a maximum depth of 6.4 m, numerous residential properties and docks along its shoreline and frequent motorized watercraft activity in the summer and snow mobile and ice fishing activity during winter months. The lake bottom is characterized by a mixture of fine (0.1 – 0.3 mm particle size, Wentworth scale), medium (0.3 – 0.5 mm,) and coarse (0.5 – 1.0 mm) sand. It is biennially stocked with walleye (*Sander vitreus*) fingerlings, but also contains a diverse array of fish species. Trials were conducted in the western portion of the lake which contained large expanses of the white water lily (*Nymphaea odorata*) in July and August.

John Lake (Fig. 1a) is a 0.8 km² wilderness lake with a maximum depth of 6.1 m located in the northeastern portion of the BWCAW and contains three designated campsites accessible by foot or nonmotorized watercraft. Motorized watercraft and power tools are prohibited in the BWCAW and access is limited by the U.S. Forest Service. The lake bottom is characterized by a mixture of coarse sand, gravel (2.0 – 4.0 mm), pebbles

(4.0 – 64.0 mm), and cobbles (64 – 256 mm) with little visible aquatic vegetation at the deployment sites.

Rush Lake (Fig. 1b) is a 1.3 km² catch-and-release private lake with a maximum depth of 86 m located roughly 2 km from the southern shore of Lake Superior and is characterized by a fine sand bottom and little aquatic vegetation. The lake is limited to Huron Mountain Club (HMC) members and Huron Mountain Wildlife Foundation researchers, and only nonmotorized watercraft are authorized.

2.1 Study Design

Fish behavior and underwater sound were monitored using an underwater video array (UVA) equipped with a Hero 5 camera (GoPro) and a SoundTrap 202 hydrophone (Ocean Instruments, NZ). The UVA was constructed out of PVC pipe (2.5 cm diameter) with a square base (41 cm per side) and a 1.2 m pipe projecting upward. The UVA was anchored to the bottom using a 1.8 kg aluminum alloy anchor and was deployed and retrieved using an attached rope/buoy. The camera was affixed to the pole 1 m vertically from the base and with the lens pointed towards the base for top-down recording. The hydrophone connected to the pole approximately 0.5 m above the base and continuously sampled underwater sounds at 24,000 Hz. A temperature sensor (Water Temp Pro v2; Hobo Instruments) was also attached to the UVA base (accuracy +/- 0.1 °C) (Fig. 2).

Both baited and unbaited trials were conducted at each lake and were designated as control, short exposure, or long exposure (Table 1). For baited trials, a mesh bait bag was attached to the base via plastic electrical ties and filled with artificial Pro Guide Formula fish attractants (in accordance with HMC restrictions). New fish attractant was

added to the bait bag prior to each baited trial and the bag was removed during unbaited trials.

All trials were conducted between sunrise and sunset (0700 to 1900 CST) at water depths between 0.6 and 2.0 m, and in areas with minimum vegetation to maximize visibility. After UVA deployment, the boat was anchored a minimum of 5 m away and an underwater speaker (UW30; Lubell Labs) was suspended 0.5 m above the lake bottom on a rope attached to the watercraft and pointed towards the UVA (Fig. 2). The speaker weighed 2.7 kg which kept the rope taught throughout deployments. The speaker was powered by a Goal Zero YETI 150 portable power station with an underwater recording of a 100 HP 2 stroke outboard motor (80 – 12,000 Hz, 130 dB) (Putland & Mensinger, 2020) transmitted from a Ruizu XO2 MP3 player.

Each trial consisted of a 15 minute pre-sound, a 25 minute experimental sound and a 20 minute post-sound period. The pre-sound period was initiated after deploying the UVA, paddling 5 m away from the site and anchoring (approximately 2 minutes after deployment). The experimental sound consisted of a short exposure of 20 seconds of motorboat sound followed by 4 minutes and 40 seconds of no sound that was repeated five times over the course of 25 minutes, or a long exposure consisting of two 5 minute sound stimuli interspersed by 15 minutes of no sound. Control trials consisted of 60 minute deployments of the apparatus without any added sound (Fig. 3). After each trial, the speaker and UVA were retrieved, and the watercraft was moved to a different deployment site a minimum of 50 m away.

2.2 Behavior/Video Analysis

All video was recorded with a GoPro camera (720 pixel resolution) at a rate of 30 frames per second and stored on a 256 GB microSD card. The viewing field of the camera was approximately 0.95 m², which included the square base, bait bag (baited trials only), and an area outside of the base (Fig. 4).

Fish behavior was initially observed frame by frame throughout the 60 minute duration of each trial using Microsoft Movies & TV (version 10.20112.10111.0) with species, number of fishes, and residence time determined. Residence time (RT) was defined as the time (in seconds) that fish remained in the field of view (FOV), which began when the anterior margin of the fish and terminus of the caudal fin were in the FOV and ceased when the entire fish was no longer in the FOV. Each fish that exited and re-entered the FOV was counted as a new individual. Observations were dominated by three fish families, Centrarchidae (sunfish), Cyprinidae (minnow), and Percidae (perch).

To determine the number of fish observed in the FOV during control trials, observations were analyzed at each minute mark. In sound trials, the time period equivalent to the sound exposure was monitored before, during and after sound exposure. During short exposure trials, where T represents the onset of the sound stimulus, two observations were taken pre-sound (T-10, T-0 sec), during sound (T+10, T+20 sec) and post-sound (T+30, T+40 sec) and in long exposure trials, observations were taken each minute pre-sound (T-4 to T-0 min), during sound (T+1 to T+5) and post-sound (T+6 to T+10 min). For RT, fish were monitored every second to calculate time to the nearest second.

2.3 Acoustic Analysis

Hydrophones sampled continuously at 24,000 Hz during each deployment. All hydrophones had a flat -3 dB frequency response between 10 and 72,000 Hz and were calibrated prior to deployment using a G.R.A.S. Type 42AA pistonphone (G.R.A.S. Sound & Vibration, Denmark) projecting 250 Hz at 114 dB re 20 μ Pa. The low frequency cut-off for underwater sound is inversely proportional to water depth (Tindle & Deane, 2005) and influenced by sediment type (Rogers & Cox, 1988; Ainslie, 2010). Owing to the shallow water depths used for trials and mixed sediment types, the minimum low frequency cut-off for analysis was 80 Hz. At frequencies above the Nyquist frequency (half the hydrophone sampling rate) signals become indistinguishable (Urlick, 1983); therefore, the maximum frequency cut-off for analysis was 12000 Hz.

Acoustic recordings were aurally and visually examined between 80 – 12000 Hz using Audacity (version 2.3.3). All acoustic data was analyzed using MATLAB software (version 2018b). The root mean squared (RMS) sound pressure levels (SPL) were calculated for every 10 seconds of recording.

2.4 Statistical Analysis

Statistical analyses were completed with R (version 4.0.3). A minimum of 10 observations per species per deployment was required for further analysis to provide a sufficient sample size for statistical analysis. Bluegill sunfish (*Lepomis macrochirus*) in Caribou lake, bluntnose minnows (*Pimephales notatus*) in John and Rush lakes, and mimic shiners (*Notropis volucellus*) and yellow perch (*Perca flavescens*) in Rush lake were sufficiently abundant in the camera field of view to allow analysis for observation number and residence time.

Control trials in Caribou, John and Rush lakes were analyzed for the number of fish observations over time using a Mann-Kendall trend test. During sound trials, the total number of fish observed 20 seconds and 5 minutes before, during and after each short and long stimulus, respectively, was not normally distributed (Shapiro-Wilk test, $p < 0.05$) and unaffected by the presence or absence of bait (Friedman's two-way ANOVA, $p > 0.05$), and therefore baited and unbaited trials were combined. The number of observations was reported as medians, 1st quartile – 3rd quartile and a Kruskal-Wallis test was performed to determine whether exposure (pre, sound, post) affected the number of fish observations, followed by a Dunn post-hoc test to determine individual effects.

Residence time was not normally distributed (Shapiro-Wilk test, $p < 0.05$). However, RT was affected by bait (Friedman's two-way ANOVA, $p < 0.05$) and values before (pre), during (sound) and after (post) each sound exposure were reported as medians, 1st quartile – 3rd quartile. A Kruskal-Wallis test was performed to determine whether exposure affected residence time in baited and unbaited trials, followed by a Dunn post-hoc test to determine individual effects.

The RMS sound pressure levels between lakes and sound exposures were analyzed using a Kruskal Wallis test followed by a Dunn post-hoc test.

R packages used included “tidyr” (version 1.1.2), “dplyr” (version 1.0.2), “TukeyC” (version 1.3-3), “FSA” (version 0.8.32), “Kendall” (version 2.2) and “PMCMR” (version 4.3).

Results

Numerous fish species were observed throughout the study (Table 2), however, four species including bluegills (Centrarchid) in Caribou lake, bluntnose minnows (cyprinid) in John and Rush lakes, and mimic shiners (cyprinid) and yellow perch (percid) in Rush lake had sufficient abundances to allow for statistical analysis.

3.1 Bait Effects

The five species that were examined in detail remained present throughout the baited and unbaited hour control trials. Bluegills appeared in relatively low numbers during the control trials with 34.2 % (baited) and 11.7% (unbaited) of the observations having these fish in the FOV. Median residence time at the baited sites was slightly but significantly greater than unbaited (12.0, 6.0 – 20.0 sec unbaited vs 13.0, 7.0 – 22.0 sec baited; Kruskal-Wallis, $\chi^2 = 8.847$, $p = 0.003$). During unbaited control trials, the number of bluntnose minnows present at each minute mark significantly decreased over time in John and Rush lakes (John, Mann-Kendall, $\tau = -0.558$, $p < 0.000$; Rush, Mann-Kendall, $\tau = -0.525$, $p < 0.000$), as well as for unbaited mimic shiners (Mann-Kendall, $\tau = -0.212$, $p = 0.022$) and baited yellow perch (Mann-Kendall, $\tau = -0.511$, $p < 0.000$) in Rush lake (Fig. 5A). Baited controls did not significantly differ over time in Caribou bluegills (Mann-Kendall, $\tau = 0.013$, $p = 0.889$), John bluntnose minnows (Mann-Kendall, $\tau = -0.049$, $p = 0.595$), Rush bluntnose minnows (Mann-Kendall, $\tau = 0.007$, $p = 0.945$) or mimic shiners (Mann-Kendall, $\tau = 0.112$, $p = 0.219$) and in unbaited controls for Caribou bluegills (Mann-Kendall, $\tau = -0.181$, $p = 0.067$) and Rush yellow perch (Mann-Kendall, $\tau = 0.034$, $p = 0.719$) (Fig. 5a).

In unbaited controls, bluegills in Caribou lake and mimic shiners (5.0, 3.0 – 9.0 sec unbaited vs 6.0, 3.0 – 11.0 sec baited; Kruskal-Wallis, $\chi^2 = 81.265$, $p < 0.000$) and

yellow perch in Rush lake (6.0, 2.0 – 12.0 sec unbaited vs 10.0, 4.0 – 17.3 sec baited; Kruskal-Wallis, $\chi^2 = 91.750$, $p < 0.000$) remained in the field of view for significantly shorter durations, but bluntnose minnows in John (5.0, 3.0 – 11.0 sec unbaited vs 4.0, 2.0 – 7.0 sec baited; Kruskal-Wallis, $\chi^2 = 197.980$, $p < 0.000$) and Rush (7.0, 3.0 – 9.0 sec unbaited vs 4.0, 3.0 – 9.3 sec baited; Kruskal-Wallis, $\chi^2 = 8.574$, $p = 0.003$) lakes had significantly longer residence times (Fig. 5b).

3.2 Soundscape

During the sound presentation, the intensity level of the short (Caribou 113.1, 109.2 – 115.9 dB re 1 μ Pa; John 125.5, 119.4 – 137.3 dB re 1 μ Pa; Rush 130.1, 118.7 – 135.9 dB re 1 μ Pa) and long (Caribou 112.8, 104.5 – 122.2 dB re 1 μ Pa; John 127.0, 115.2 – 133.9 dB re 1 μ Pa; Rush 130.8, 118.0 – 134.4 dB re 1 μ Pa) trials were significantly greater than ambient sound (in the absence of motorized water craft) SPLs in Caribou (77.2, 74.8 – 78.0 dB re 1 μ Pa; Kruskal-Wallis, $\chi^2 = 27.9$, $p < 0.001$), John (77.4, 74.3 – 81.9 dB re 1 μ Pa; Kruskal-Wallis, $\chi^2 = 28.8$, $p < 0.001$) and Rush (78.1, 72.7 – 95.7 dB re 1 μ Pa; Kruskal-Wallis, $\chi^2 = 32.6$, $p < 0.001$). However, ambient SPLs did not significantly differ between lakes (Kruskal-Wallis, $\chi^2 = 0.4$, $p = 0.800$) (Fig. 6).

3.3 Fish Presence

Table 2 shows the median number of fish observed during the trials for each lake. The percent abundance of fish observed in each lake was calculated by dividing the number of each species by the total number of fish observed per lake. Bluntnose minnows and northern pike (*Esox lucius*) were observed in each lake, yellow perch and blacknose shiners (*Notropis heterolepis*) were found in Caribou and Rush lakes, and smallmouth bass (*Micropterus dolomieu*) were seen in John and Rush lakes. Common

shiner (*Notropis cornutus frontalis*), mimic shiner, logperch (*Percina caprodes semifasciata*), northern rockbass (*Ambloplites rupestris*), and mottled sculpin (*Cottus bairdii*) were observed solely in Rush lake with bluegills, largemouth bass (*Micropterus salmoides*), and black crappie (*Pomoxis nigromaculatus*) observations restricted to Caribou lake (Table 2). Bluegills, bluntnose minnows and mimic shiners dominated the FOV and comprised of 74.6%, 99.8% and 43.2% of the fish observed in Caribou, John and Rush lakes, respectively. The three most common families observed were Cyprinidae (14.7% Caribou, 99.8% John, 60.2% Rush), Centrarchidae (79.7 % Caribou, 0.2% John, 0.5% Rush) and Percidae (5.5% Caribou, 39.1% Rush).

The number of fish observed in the three experimental periods (pre, sound, post) did not significantly differ between baited and unbaited trials in any of the species (Friedman two-way ANOVA, $p > 0.05$) and trials for each lake were combined. Caribou lake bluegills and Rush lake bluntnose minnows saw no significant difference the number observed before, during and after the sound exposure (Kruskal-Wallis, $p > 0.05$).

The short sound exposures significantly decreased the median number of bluntnose minnows observed during the sound stimuli (1.0, 0.0 – 5.0 pre vs 0.0, 0.0 – 3.0 sound) in John Lake (Kruskal-Wallis, $\chi^2 = 49.299$, $p = 0.032$), but did not significantly change the number of mimic shiners or yellow perch observed in Rush lake (Kruskal-Wallis, $\chi^2 = 49.299$, $p > 0.05$) (Fig. 7). The long sound exposures also did not significantly change the number of fish in the field of view before, during and after the stimulus in John or Rush lakes (Kruskal-Wallis, $\chi^2 = 231.920$, $p > 0.05$) (Fig. 7).

3.4 Residence Time

The effect of sound on residence time (the duration fish remained in the field of view) was quantified by species during short and long sound trials. Residence times were often significantly different between baited and unbaited deployments and each type was examined independently.

Caribou – Bluegills

Bluegills in Caribou lake responded to short and long duration sound in unbaited trials as evidenced by significantly altered residence time. The median residence time for bluegills significantly decreased from pre (11.0, 7.0 – 23.0 sec) to sound (7.0, 5.0 – 12.0 sec) (Kruskal-Wallis, $\chi^2 = 20.392$, $p = 0.003$) in unbaited short exposure trials, but significantly increased from pre (9.0, 6.0 – 15.0 sec) to sound (15.0, 9.0 – 29.8 sec) in unbaited long exposure trials (Kruskal-Wallis, $\chi^2 = 89.743$, $p < 0.000$) (Fig. 8b). Bluegills in baited trials did not display any changes to sound exposures (short, Kruskal-Wallis, $\chi^2 = 20.392$, $p > 0.050$; long, Kruskal-Wallis, $\chi^2 = 89.743$, $p > 0.050$) (Fig. 8a).

John – Bluntnose Minnows

Bluntnose minnows in John lake significantly decreased their residence time from pre (short, 6.0, 4.0 – 7.0 sec; long, 5.0, 3.0 – 7.0 sec) to sound (short, 5.0, 3.0 – 7.0 sec; long, 4.0, 3.0 – 6.0 sec) during baited short (Kruskal-Wallis, $\chi^2 = 3720.900$, $p < 0.000$) and long (Kruskal-Wallis, $\chi^2 = 211.040$, $p < 0.000$) sound exposures whereas they significantly increased residence time from pre (4.0, 3.0 – 5.0 sec) to sound (6.0, 3.0 – 8.0 sec) in response to unbaited long sound exposures (Kruskal-Wallis, $\chi^2 = 211.040$, $p < 0.000$) (Fig. 8). Bluntnose minnows returned to pre-sound residence times following both baited (4.0, 3.0 – 8.0 sec) and unbaited (4.0, 2.0 – 6.0 sec) long sound exposures (Fig. 8).

Rush – Bluntnose Minnows, Mimic Shiners, Yellow Perch

Bluntnose minnows, mimic shiners and yellow perch responded strongly to sound in baited trials but showed little reaction in the absence of bait. Each species significantly increased their residence time from pre (bluntnose minnows, 5.0, 3.0 – 8.0 sec; mimic shiners, 5.0, 3.0 – 10.0 sec; yellow perch, 6.0, 3.0 – 10.0 sec) to sound (bluntnose minnows, 11.0, 4.0 – 22.0 sec; mimic shiners, 9.0, 4.0 – 22.3 sec; yellow perch, 13.0, 6.0 – 33.8 sec) in baited short exposure trials (bluntnose minnows, Kruskal-Wallis, $\chi^2 = 103.040$, $p < 0.000$; mimic shiners, Kruskal-Wallis, $\chi^2 = 319.100$, $p < 0.000$; yellow perch, Kruskal-Wallis, $\chi^2 = 298.440$, $p < 0.000$), but did not return to pre-sound residence times in the post exposure period (Fig. 8a). During the long sound exposure, only mimic shiners significantly changed residence times from pre (baited, 3.0, 2.0 – 8.0; unbaited, 2.0, 1.0 – 3.0 sec) to sound (baited, 6.0, 2.0 – 12.0 sec; unbaited 3.0, 1.0 – 6.0 sec) in baited and unbaited trials (baited, Kruskal-Wallis, $\chi^2 = 449.240$, $p < 0.000$; unbaited, Kruskal-Wallis, $\chi^2 = 449.240$, $p = 0.013$) with no difference observed for bluntnose minnows and yellow perch residence times (bluntnose minnows, Kruskal-Wallis, $\chi^2 = 103.040$, $p > 0.050$; mimic shiners, Kruskal-Wallis, $\chi^2 = 319.190$, $p > 0.050$) or long (bluntnose minnows, Kruskal-Wallis, $\chi^2 = 9.256$, $p > 0.050$; mimic shiners, Kruskal-Wallis, $\chi^2 = 449.240$, $p > 0.050$) sound exposures (Fig. 8b).

Discussion

Both baited and unbaited UVAs successfully attracted fish quickly after deployment and maintained fish presence throughout each trial. However, anthropogenic sound had little effect on the number of fish with only bluntnose minnows in John Lake significantly reducing their numbers during short sound exposures. Wider variation was observed in residence time with several species increasing time around the apparatus

during sound presentations. While in situ field trials can provide the most accurate assessment of behavioral changes induced by anthropogenic sound, these experiments detailed the challenges of inter lake comparisons with multiple species.

Numerous reviews have called for more in-situ studies on the effects of anthropogenic sound on aquatic animals (Popper & Hawkins, 2019) and this study represents one of the first attempts to compare the effects of anthropogenic sound on wild fishes that are routinely exposed to sound in both summer and winter versus wilderness populations that have not been exposed for multiple generations. The UVA minimized interference from divers or boats, however, bait is often needed to attract sufficient fishes for analysis which may impact animal behavior. This study found that the three dimensional structure was moderately successful in attracting fish without bait, although the presence of bait increased fish number and residence times during the control deployments.

The lakes originally were chosen based on similar species composition to allow comparison between the same fishes in recreational and wilderness lakes. The UVA was able to monitor fish responses throughout the deployments, however high variability was seen both at the individual and species levels that would enter the FOV. For example, large predatory fish (northern pike) and benthic species were rarely observed and therefore analysis was limited to fishes that appeared in sufficient numbers.

Since attractants could influence how fish reacted to stimuli, both baited and unbaited trials were performed. Bait minimally affected the total number of fish observations, however, individuals in baited trials were more likely to increase their residence time in response to longer sound stimuli. In both baited and unbaited trials,

otophysan fish, including bluntnose minnows and mimic shiners of the cyprinid family, seemed more responsive to sound than non-otophysan fish, which aligns with previous studies that found fish with higher hearing sensitivities experienced greater decreases in foraging when compared to individuals with lower hearing sensitivities (Magnhagen et al., 2017; Pieniazek et al., 2020). Cyprinids were affected by motorboat sound in all lakes, evident in numerous changes in residence time especially in John lake and were observed in UVA trials more frequently in wilderness lakes. Cyprinids may also be more susceptible to boat sound pollution compared to bluegills (centrarchids) and yellow perch (percids) that lack specialized ancillary structures and have lower hearing sensitivities (Popper & Fay, 2011).

Interestingly, anthropogenic activity on the lake may have led to more cautious behavior that deterred individuals from entering the FOV, although the techniques could not determine if this was due to different species compositions, abundance or fishing pressure. Similar results have been observed in areas of reduced fishing pressure where inhabitants were considered “bolder” and approached structures or new objects more readily (Mensing et al., 2018).

Caribou Lake

Fewer fish were observed in Caribou lake compared to the wilderness lakes and observations were dominated by bluegill sunfish (~80%), which are medium sized, omnivorous predatory fish with larger individuals targeted for human consumption. Bluegill hearing sensitivity ranges from 300 – 2000 Hz and are not known to have any hearing specializations (Scholik & Yan, 2002). Bluegill numbers remained constant during the sound exposure although residence time declined slightly during short and

increased during the long exposure trials for unbaited deployments. Bluegill behavior may have been influenced by several seasonal factors. The 2020 pandemic delayed deployment until mid-summer and at that time, seasonal aquatic vegetation growth reduced the sound intensity at the UVA site. Additionally, the study overlapped with bluegills nesting season, with many male sunfish exhibiting aggressive intra and interspecific behavior correlated with rim circling nest guarding, and territoriality (Colgan et al., 1979) which deterred other fish from approaching the set up and resulted in a low species diversity. A concurrent study in another recreation lake, focused exclusively on nest guarding sunfish, showed minimal impact of sound on behavior (Mensing, unpublished). Unfortunately, insufficient numbers of centrarchids were seen in wilderness lakes to make comparisons to their behavior in public access lakes.

John Lake

The BWCAW was established in 1978 and therefore fish in John lake have not been exposed to motorized sounds for over 40 years. Smallmouth bass and northern pike are the primary game fish in this lake, but were rarely seen, and observations were dominated by bluntnose minnows, an otophysan which preys on aquatic insects, algae, diatoms, aquatic insect larvae, and small crustaceans and are not targeted for fishing. Their hearing range is comparable to another otophysan fish, the fathead minnow (*Pimephales promelas*), which ranges between 300 – 4000 Hz due to ancillary hearing structures (Scholik & Yan, 2002). Sustained sound had no effect on bluntnose minnows suggesting that the fish may quickly habituate to sound.

Rush Lake

Rush lake is contained within the property of the Huron Mountain Club, which has been a protected, private club since 1890 and access is limited to club members (~50 per year) and field researchers and sustains little if any angling pressure. A diverse array of fish species was observed throughout this study, with observations dominated by bluntnose minnows, mimic shiners, and yellow perch. Mimic shiners are small, otophysan fish that consume mostly *Daphnia*, are an important food source for larger predatory fish and have similar hearing sensitivities to bluntnose minnows (Gorski et al., 1999). Yellow perch are medium size predatory fish as adults and are often targeted by anglers. Their hearing sensitivities are comparable to European perch which ranges between 100 – 1000 Hz, with best sensitivity at 200 – 300 Hz (Magnhagen et al., 2017). Bluntnose minnows, mimic shiners and yellow perch showed little change in overall numbers in response to sound which may have been attributed to longer duration residence times during sound exposures in baited trials. Additionally, since sound did not affect the number of fish in Rush lake, these results contrast with the other wilderness lake in which short duration sound decreased fish numbers.

This study is one of the first to compare freshwater fish behavior between recreational and wilderness lakes. Somewhat surprisingly, there were only small differences noted which may have been attributed to several aspects of the study. Although the lakes were chosen based on similar species composition, the study was limited to the fish species that were in view during the sound presentations and temporal variations prevented multiple species comparisons between lakes. The 2020 pandemic delayed the initiation of field studies until mid-June, which corresponded with the bluegill mating season and an increase in aquatic vegetation. Additionally, regulations

prevented the use of motorized watercraft in wilderness lakes which made it impossible to recreate the exact sound, visual presence, and water disturbance that fish would normally experience during motorized watercraft passage. This may be especially important as the fish inner ear and external lateral line respond to particle motion as well as sound pressure and the use of a static speaker differs from a moving sound source.

Bluegills in Caribou lake showed less reaction to sound than cyprinids in the wilderness lakes, which may have been due to habituation from frequent boat sound exposure (Jacobsen et al., 2014; Johansson et al., 2016; Magnhagen et al., 2017), long term hearing impairment that decreased hearing sensitivities (Scholik & Yan, 2001; Scholik & Yan, 2002; Popper et al., 2003; Popper & Hastings, 2009; Mickle & Higgs, 2017) or territorial behavior. It is also thought that this was the first anthropogenic sound exposure to the wilderness populations and these fishes remained relatively indifferent to sound stimuli despite the minnows and shiners having greater hearing sensitivity than bluegill and yellow perch. However, the wilderness fish were not routinely targeted for angling and received less fishing pressure than individuals in the recreational lake, which is consistent with bolder behavior to novel stimuli exhibited by fish in protected reserves (Mensing et al., 2018, Nunes et al., 2018)

Numerous studies have demonstrated that cyprinids are more susceptible to behavioral changes such as decreased foraging, presence, and hearing sensitivity in response to anthropogenic sound (Scholik & Yan, 2001; Scholik & Yan, 2002; Magnhagen et al., 2017; Pieniazek et al., 2020). This study found that residence time in wild, free swimming cyprinids greatly varied in pre, sound and post exposure periods in nearly all trials.

Summary

Overall, this study demonstrated that underwater video provides a mechanism for analyzing the effects of anthropogenic sound on freshwater fish behavior in public and wilderness lakes. Wilderness lakes present relatively pristine environments and serve as a reference for in-situ fish behavior with minimal human activity. These methods can be applied to a variety of species in different freshwater habitats to improve knowledge and understanding of the impact of sound on freshwater ecosystems, which is fundamental to both ecological insight and conservation efforts. However, the study was limited by the inability to recruit similar fish species for direct comparisons between the lakes. Future studies may want to use large cages or enclosures to ensure the fish remain within observable distances while still allowing movement to sound. Utilizing these methods may ultimately lead to a better understanding of aquatic organisms and environments they inhabit.

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Tables and Figures

Table 1. Deployments in each lake. Numbers indicate the baited/unbaited deployments for each lake and trial type.

	Control	Short	Long
Caribou	6/6	6/6	6/7
John	5/5	5/5	5/5
Rush	6/8	6/6	5/6

Table 2. Number of fish observed per trial. Values represent the median \pm IQR number of fishes, with numbers in parentheses (#) indicating the number of trials in which each species was present. Dashes indicate no observations.

Family	Species	Caribou			John			Rush		
		Control	Short	Long	Control	Short	Long	Control	Short	Long
Centrarchidae	Black Crappie	0.5 \pm 2.5 (6)	0.5 \pm 1.3 (6)	1.0 \pm 1.0 (7)	-	-	-	-	-	-
	Bluegill	34.5 \pm 69.5 (12)	35.5 \pm 41.5 (12)	48.0 \pm 37.0 (13)	-	-	-	-	-	-
	Largemouth Bass	0.0 \pm 0.0 (1)	-	4.0 \pm 12.0 (9)	-	-	-	-	-	-
	Smallmouth Bass	-	-	-	0.0 \pm 0.0 (2)	0.0 \pm 0.0 (1)	0.0 \pm 0.0 (2)	1.0 \pm 3.8 (8)	0.0 \pm 0.3 (3)	1.0 \pm 1.0 (8)
	N. Rock Bass	-	-	-	-	-	-	0.0 \pm 0.0 (2)	0.0 \pm 17.0 (5)	0.0 \pm 0.0 (2)
	N. Mottled Sculpin	-	-	-	-	-	-	0.0 \pm 0.0 (3)	0.0 \pm 0.5 (3)	0.0 \pm 1.5 (4)
Cyprinidae	Bluntnose Minnow	4.5 \pm 10.5 (10)	2.5 \pm 5.8 (10)	0.0 \pm 1.0 (4)	345.0 \pm 810.3 (9)	1643.5 \pm 2364.5 (10)	653.5 \pm 1205.3 (10)	16.5 \pm 32.5 (11)	26.0 \pm 23.0 (11)	19.0 \pm 83.0 (9)
	Blacknose Shiner	-	0.0 \pm 5.3 (5)	1.0 \pm 5.0 (7)	-	-	-	0.0 \pm 0.0 (2)	-	-
	Common Shiner	-	-	-	-	-	-	0.0 \pm 2.8 (6)	0.0 \pm 0.0 (1)	0.0 \pm 0.0 (2)
	Mimic Shiner	-	-	-	-	-	-	204.5 \pm 359.5 (11)	20.5 \pm 102.8 (9)	12.0 \pm 320.0 (8)
Esocidae	Northern Pike	0.0 \pm 0.0 (1)	-	0.0 \pm 0.0 (1)	-	0.0 \pm 0.0 (2)	0.0 \pm 1.0 (4)	0.0 \pm 0.0 (2)	0.0 \pm 0.0 (2)	-
Percidae	Yellow Perch	5.0 \pm 6.0 (10)	0.0 \pm 1.0 (4)	1.0 \pm 3.0 (7)	-	-	-	40.5 \pm 128.0 (14)	149.0 \pm 454.0 (12)	47.0 \pm 57.0 (11)

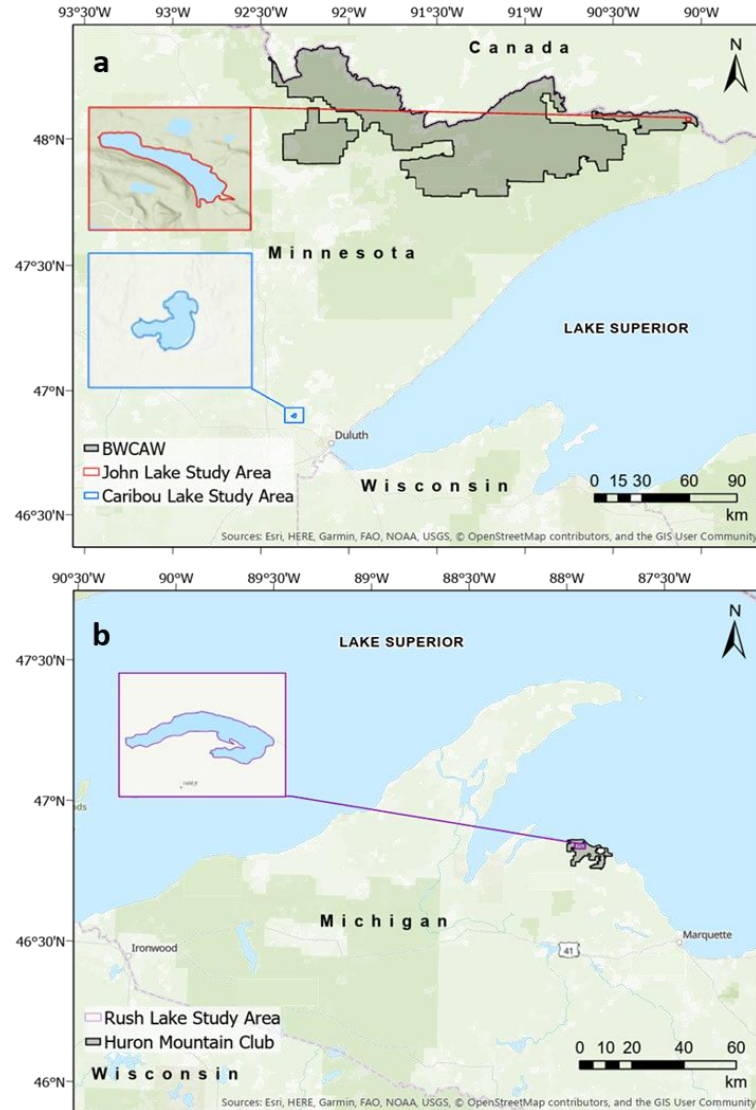


Figure 1. Study sites at (a) Caribou Lake and John Lake in northern Minnesota and (b) Rush Lake in the Upper Peninsula of Michigan. Maps were created in ArcGIS Pro (version 2.7.0) using shapefiles obtained from the Huron Mountain Wildlife Foundation, the Minnesota Department of Natural Resources, and the Metropolitan Council.

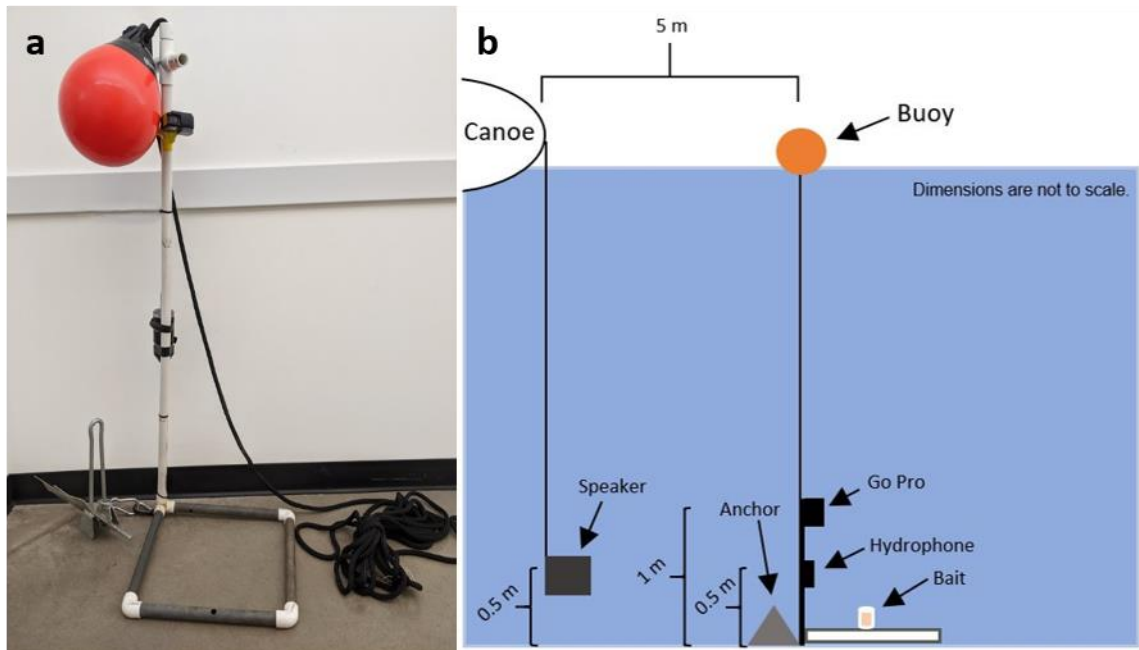


Figure 2. (a) Photograph of the underwater video array and (b) diagram of its deployment with the underwater speaker deployed 5 m away from the UVA. The square base measured 41 cm x 41 cm.

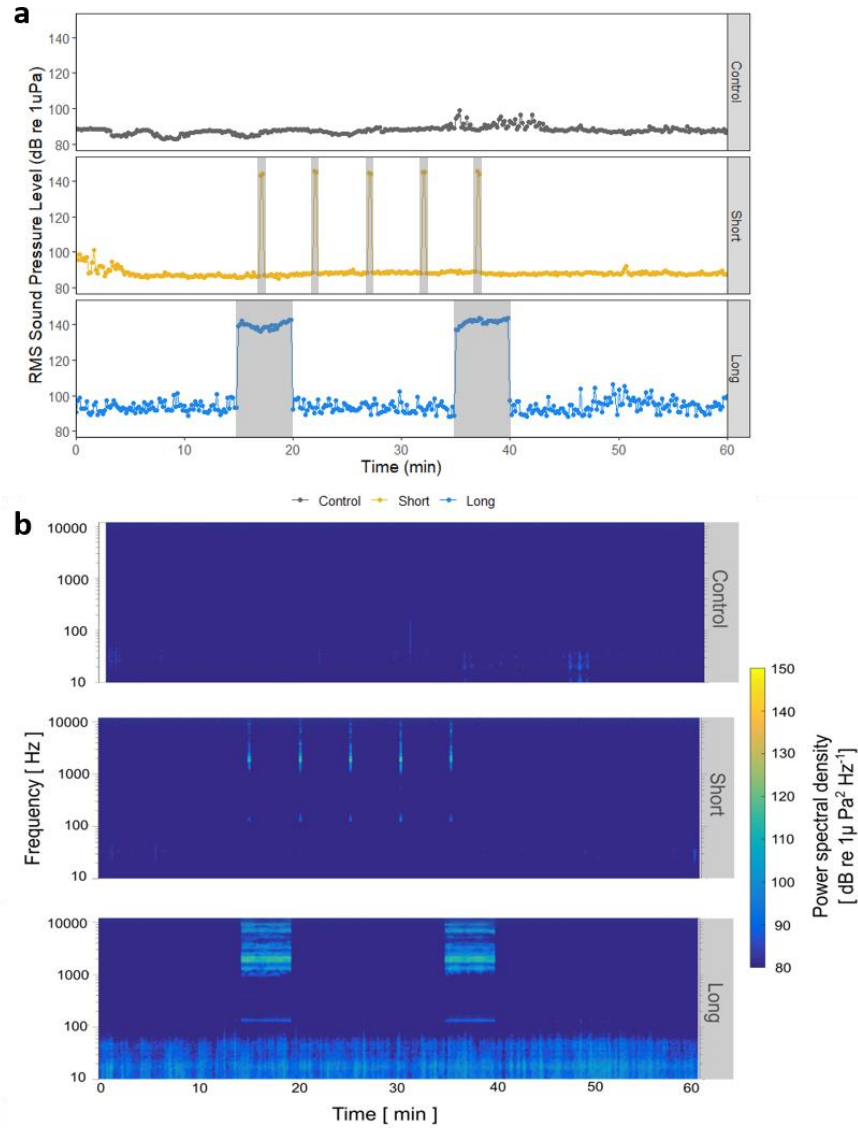


Figure 3. Sound recordings. (a) The root mean squared (RMS) sound pressure levels (SPLs) (dB re 1 μ Pa) and (b) the spectrogram of baited trials in John Lake is plotted against time. The RMS SPLs and spectrogram for a control (top panels), short sound exposure (middle) and long sound exposure (bottom) trial are plotted against time. The SPLs were determined every 10 seconds with data points joined by lines for illustrative purposes. The gray boxes represent the motorboat sound stimuli periods (a) and the color scale shows power spectral density (b).



Figure 4. Single frame from the underwater video array (UVA) camera. The apparatus was deployed in 1.5 m depth during an unbaited control trial at Rush Lake. The PVC square base measured 41 cm x 41 cm for scale and one yellow perch is circled.

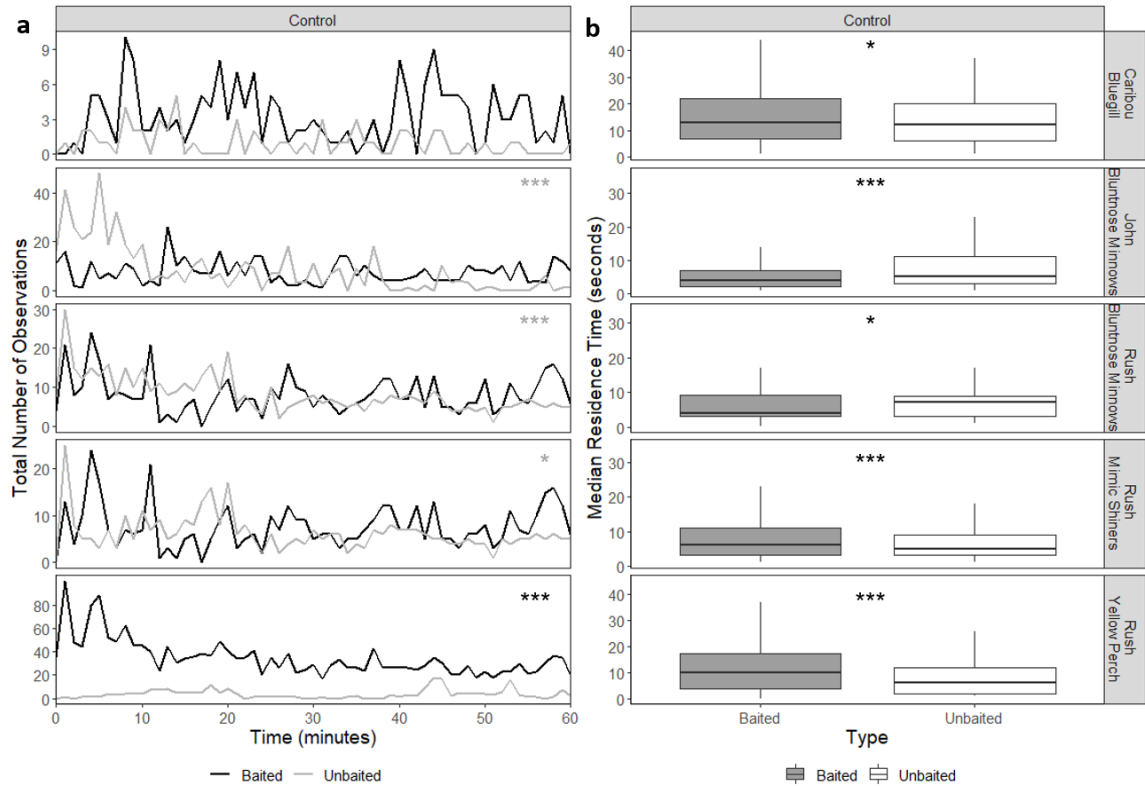


Figure 5. (a) Total number of fish present at each minute mark and (b) median residence time (seconds) of species during baited and unbaited controls in each lake. Baited (black) and unbaited (gray) trials represent the total number of fish per species in the FOV at each minute mark throughout the 60 minute control trials (a) and the median residence time (b) per species for bluegills in Caribou ($n = 6$ baited control, $n = 6$ unbaited control), bluntnose minnows in John ($n = 5$ baited control, $n = 5$ unbaited control) and bluntnose minnows, mimic shiners, and yellow perch in Rush ($n = 6$ baited control, $n = 8$ unbaited control) lakes. Box and whiskers represent the median \pm IQR and the minimum/maximum, respectively. A Mann-Kendall test was used to determine upward or downward trends in the total number of fish (a) and a Kruskal-Wallis test was used to determine the effect of bait on residence time (b) (* $p = 0.010$, ** $p = 0.001$, *** $p < 0.001$).

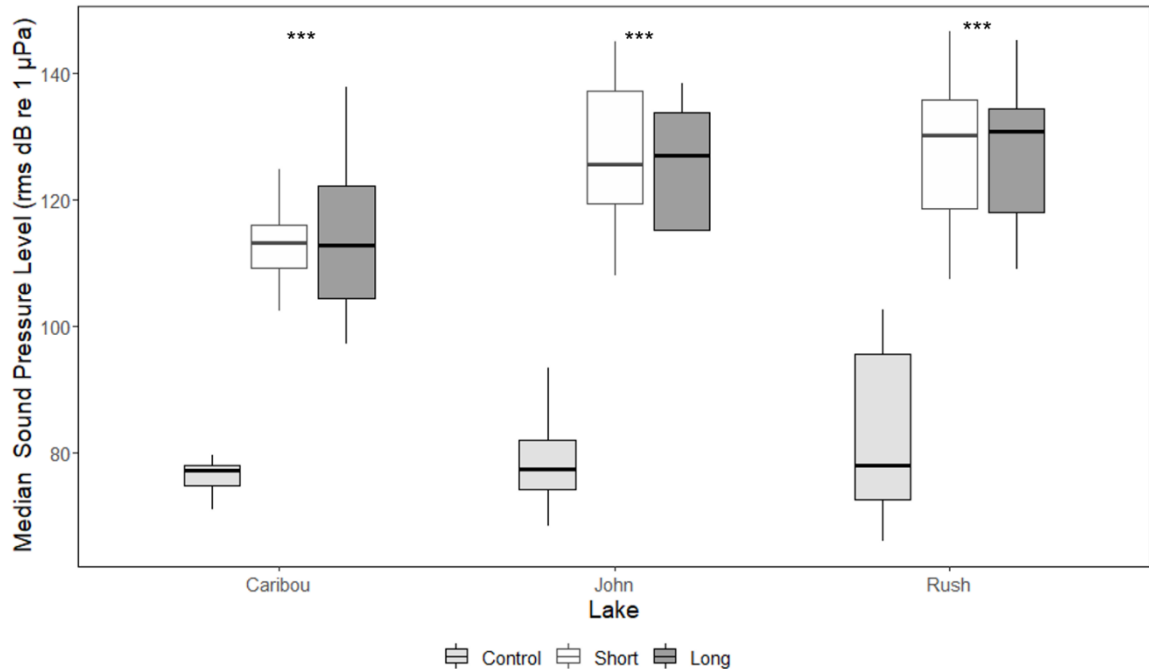


Figure 6. Median root mean squared (RMS) sound pressure levels (SPLs) (dB re 1 μ Pa) recorded every 10 seconds during controls and sound trials in each lake. Control bars (light gray) represent the median ambient SPLs for both baited and unbaited control trials in Caribou), John and Rush lakes. Short (20 second intervals, white) and long (5 minute intervals, dark gray) bars represent the median SPLs during the motorboat sound stimulus for both baited and unbaited sound trials in Caribou John and Rush) lakes. Box and whiskers represent the median \pm IQR and the minimum/maximum, respectively. A Kruskal-Wallis test determined significant differences, marked by asterisk(s) (***) $p < 0.001$), between motorboat sound stimuli (short and long) compared with controls in each lake.

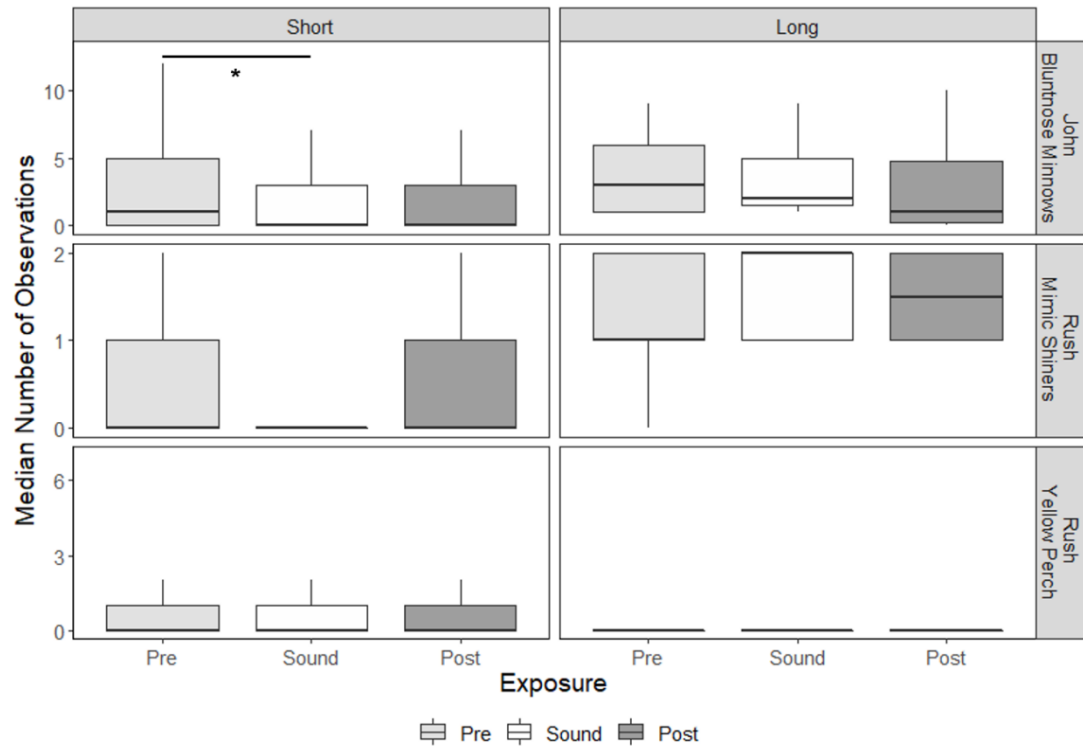


Figure 7. Median number of fish observed before, during and after the short and long sound stimuli in John (bluntnose minnows) and Rush (mimic shiners and yellow perch) lakes. Light gray (pre), white (sound) and dark gray (post) bars represent the median number of bluntnose minnows in John ($n = 10$ short, $n = 8$ long) and mimic shiners ($n = 8$ short, $n = 6$ long) and yellow perch ($n = 12$ short, $n = 11$ long) in Rush lakes in the FOV every 10 seconds for 20 seconds and every minute for 5 minutes prior to, during, and after the sound stimuli for short and long sound trials, respectively. Box and whiskers represent the median \pm IQR and the minimum/maximum, respectively. A Kruskal-Wallis was used to test for significant differences, marked by an asterisk(s) (* $p = 0.010$), between each stage of the exposure (pre, sound, post).

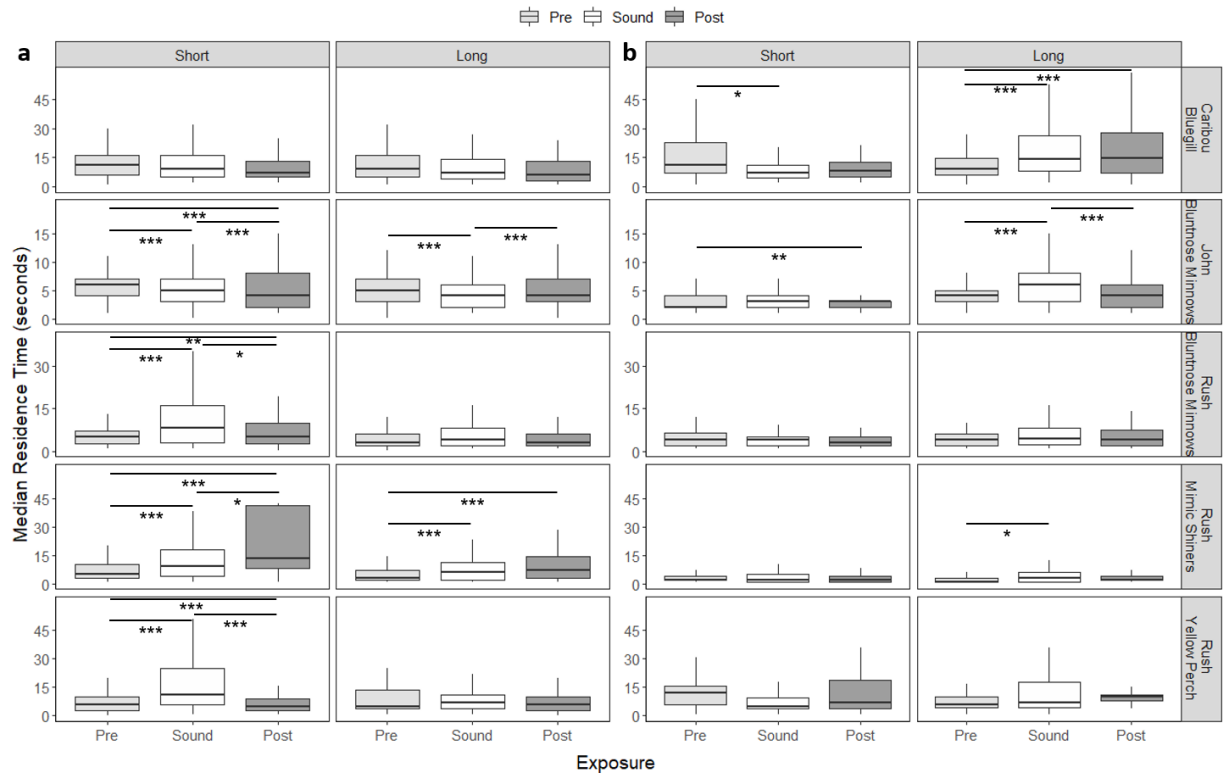


Figure 8. Median residence time (seconds) in each lake during baited (a) and unbaited (b) short and long sound trials. The median residence time per species for bluegills in Caribou (n = 12 short, n = 13 long), bluntnose minnows in John (n = 10 short, n = 10 long) and bluntnose minnows, mimic shiners, and yellow perch in Rush (n = 12 short, n = 11 long) lakes during baited and unbaited trials for 15 minutes prior to (pre, light gray), during (sound, white) and 20 minutes after (post, dark gray) the sound stimulus period for short and long trials. Box and whiskers represent the median \pm IQR and the minimum/maximum, respectively. A Kruskal-Wallis was used to test for significant differences, marked by asterisk(s) (* p = 0.010, ** p = 0.001, *** p < 0.001), between each stage of the exposure (pre, sound, post), marked by lines.

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